

**LISTING OF THE CLAIMS**

1. (Original) A method for producing carotenoids or their precursors using genetically modified organisms of the *Blakeslea* genus, which method comprises the following steps:
  - (i) transformation of at least one of the cells,
  - (ii) homokaryotic conversion of the cells obtained in step (i) to produce cells in which one or more genetic characteristics of the nuclei are all modified in an identical manner and said genetic modification manifests itself in the cells, and
  - (iii) selection and reproduction of the genetically modified cell or cells,
  - (iv) cultivation of the genetically modified cells,
  - (v) preparation of the carotenoid produced by the genetically modified cells or the carotenoid precursor produced by said genetically modified cells.
2. (Original) The method according to claim 1, wherein the cells are from fungi of the *Blakeslea trispora* species.
3. (Previously presented) The method according to claim 1, wherein a vector or free nucleic acids are used in the transformation of step (i).
4. (Previously presented) The method according to claim 3, wherein the vector employed in the transformation is integrated into the genome of at least one of the cells.
5. (Previously presented) The method according to claim 4, wherein the vector employed in the transformation comprises a promoter and/or a terminator.
6. (Previously presented) The method according to claim 3, wherein a vector comprising a gpd, pcarB, pcarRA and/or ptef1 promoter and/or a trpC terminator is employed in the transformation.
7. (Previously presented) The method according to claim 3, wherein a vector comprising a resistance gene is employed in the transformation.

8. (Previously presented) The method according to claim 7, wherein the vector employed in the transformation comprises a hygromycin resistance gene (hph).
9. (Previously presented) The method according to claim 6, wherein the gpd promoter comprises the sequence SEQ ID NO: 1.
10. (Previously presented) The method according to claim 6, wherein the trpC terminator comprises the sequence SEQ ID NO: 2.
11. (Previously presented) The method according to claim 6, wherein the ptef1 promoter comprises the sequence SEQ ID NO: 35.
12. (Previously presented) The method according to claim 6, wherein the gpd promoter and the trpC terminator are derived from *Aspergillus nidulans*.
13. (Previously presented) The method according to claim 3, wherein the vector comprises the sequence SEQ ID NO: 3.
14. (Previously presented) The method according to claim 1, wherein the transformation is carried out using agrobacteria, conjugation, chemicals, electroporation, bombardment with DNA-loaded particles, protoplasts or microinjection.
15. (Previously presented) The method according to claim 1, wherein a mutagenic agent is employed in the homokaryotic conversion of step (ii).
16. (Original) The method according to claim 15, wherein the mutagenic agent employed is N-methyl-N'-nitronitrosoguanidine (MNNG), UV radiation or X rays.
17. (Previously presented) The method according to claim 1, wherein the selection is carried out by labeling and/or selecting the mononuclear cells.
18. (Previously presented) The method according to claim 1, wherein 5-carbon-5-deazariboflavin (darf) and hygromycin (hyg) or 5-fluororotate (FOA) and uracil and hygromycin are employed in the selection.

19. (Previously presented) The method according to claim 3, wherein the vector employed in the transformation includes genetic information for producing carotenoids or their precursors.
20. (Previously presented) The method according to claim 3, wherein the vector employed in the transformation includes genetic information for producing carotenes or xanthophylls.
21. (Previously presented) The method according to claim 3, wherein the vector employed in the transformation includes genetic information for producing astaxanthin, zeaxanthin, echinenone,  $\beta$ -cryptoxanthin, andonixanthin, adonirubin, canthaxanthin, 3-hydroxyechinenone, 3'-hydroxyechinenone, lycopene,  $\beta$ -carotene,  $\alpha$ -carotene, lutein, phytofluene, bixin or phytoene.
22. (Previously presented) A method for providing at least one highly pure carotenoid and a foodstuff comprising carotenoid-producing organisms and at least the one carotenoid, which method comprises, after cultivation of carotenoid-producing genetically modified organisms of the *Blakeslea* genus according to claim 1, the following steps:
  - I) removal of the biomass,
    - IA) optional washing of the biomass with a solvent in which carotenoids are not soluble, in particular water,
    - IB) sterilization and cell disruption of the biomass,
    - IC) optional drying and/or homogeneous distribution, and
  - II) partial extraction of the carotenoids from the disrupted biomass by means of a carotenoid-dissolving solvent and separation of said solvent from said biomass,
    - IIA)
      - 1) removal of residual solvent from the carotenoid-containing biomass,
      - 2) optional homogeneous suspension of the biomass, with a biomass solid content of > 2% and < 50%,
      - 3) drying of the biomass or suspension for producing the foodstuff,
    - IIB)

- 1) crystallization of the carotenoids from the solvent used and isolation of the carotenoid crystals, in particular by filtration.
23. (Original) The method according to claim 22, wherein the at least one carotenoid is selected from the group consisting of carotenes and xanthophylls.
24. (Previously presented) The method according to claim 22, wherein the at least one carotenoid is selected from the group consisting of astaxanthin, zeaxanthin, echinenone,  $\beta$ -cryptoxanthin, andonixanthin, adonirubin, canthaxanthin, 3-hydroxyechinenone, 3'-hydroxy-echinenone, lycopene,  $\beta$ -carotene, lutein, phytofluene, bixin and phytoene.
25. (Previously presented) The method according to claim 22, wherein the at least one carotenoid is astaxanthin, zeaxanthin, bixin or phytoene.
26. (Previously presented) The method according to claim 22, wherein sterilization and cell disruption are carried out using steam or microwave radiation.
27. (Previously presented) The method according to claim 22, wherein the carotenoids are extracted from the biomass using dichloromethane or supercritical carbon dioxide or tetrahydrofuran.
28. (Original) The method according to claim 27, wherein the carotenoids dissolved in supercritical carbon dioxide are isolated directly or are taken up in dichloromethane.
29. (Previously presented) The method according to claim 22, wherein the carotenoids are extracted from the biomass in a one-stage or, if appropriate, multistage process.
30. (Previously presented) The method according to claim 22, wherein solvents are removed from the biomass in step IIA1) using steam distillation.
31. (Previously presented) The method according to claim 22, wherein drying in step IIA3) is carried out using spray drying or contact drying.
32. (Previously presented) The method according to claim 22, wherein crystallization in step IIB1) is carried out by replacing the solvent gradually with a solvent in which carotenoids are not soluble.

33. (Previously presented) The method according to claim 32, wherein the solvent used is replaced with water or with a lower alcohol.
34. (Previously presented) The method according to claim 13, wherein the genetically modified organism of the Blakeslea genus can be produced by transformation with a vector which comprises a sequence selected from the group consisting of SEQ ID NOs: 37 – 51 and SEQ ID NO: 62.
35. (Previously presented) A method for producing a foodstuff comprising organisms of the Blakeslea genus and at least one carotenoid, which method comprises, after cultivation of carotenoid-producing genetically modified organisms of the Blakeslea genus according to claim 1, the following steps:
  - I) homogeneous suspension of the solids of the culture broth,  
and
  - IIA) for a biomass solid content of the culture broth of > 2%:
    - 1) optional concentration of the culture broth to give a solid content of < 50%, and
    - 2) drying of the culture broth to produce the foodstuff,  
or
  - IIB) for a solid content of < 2% of the culture broth,
    - 1) concentration of the culture broth to give a solid content of > 2% and < 50%, and
    - 2) drying of the suspension to produce the foodstuff,  
or
  - IIC) independently of the solid content of the culture broth,
    - 1) removal of the biomass,
    - 2) optional washing of the biomass with solvents in which carotenoids are not soluble, in particular water,
    - 3) sterilization and cell disruption,
    - 4) optional drying and homogeneous distribution,
    - 5) partial extraction of the carotenoids from the biomass using a carotenoid-dissolving solvent,

- 5a) removal of the carotenoid-containing biomass from the carotenoid-containing solvent,
- 5b) removal of residual solvent from the biomass, and
- 5c) drying of the biomass to produce the foodstuff,

- 6) crystallization of the carotenoids from the solvent used in 5a) and isolation of the carotenoid crystals, in particular by filtration.

36. (Original) The method according to claim 35, wherein the at least one carotenoid is selected from the group consisting of carotenes and xanthophylls.

37. (Previously presented) The method according to claim 35, wherein the at least one carotenoid is selected from the group consisting of astaxanthin, zeaxanthin, echinenone,  $\beta$ -cryptoxanthin, andonixanthin, adonirubin, canthaxanthin, 3-hydroxyechinenone, 3'-hydroxyechinenone, lycopene,  $\beta$ -carotene, lutein, bixin and phytoene.

38. (Previously presented) The method according to claim 35, wherein the at least one carotenoid is astaxanthin, zeaxanthin, bixin or phytoene.

39. (Previously presented) The method according to claim 35, wherein sterilization and cell disruption in step IIC3) are carried out using steam or microwave radiation.

40. (Previously presented) The method according to claim 35, wherein the carotenoids are extracted from the biomass in step IIC5) using dichloromethane or supercritical carbon dioxide.

41. (Original) The method according to claim 40, wherein the carotenoids dissolved in supercritical carbon dioxide are isolated directly or are taken up in dichloromethane.

42. (Previously presented) The method according to claim 35, wherein the carotenoids are extracted from the biomass in a one-stage or, if appropriate, multistage process.

43. (Previously presented) The method according to claim 35, wherein solvents are removed from the biomass in step IIC5b) using steam distillation.

44. (Previously presented) The method according to claim 35, wherein drying in any of steps IIA2), IIB2) and IIC5c) is carried out using spray drying or contact drying.

45. (Previously presented) The method according to claim 35, wherein crystallization in step IIC6) is carried out by replacing the solvent gradually with a solvent in which carotenoids are not soluble.
46. (Previously presented) The method according to claim 45, wherein the solvent used is replaced with water or with a lower alcohol.
47. (Previously presented) The method according to claim 35, wherein the genetically modified organism of the Blakeslea genus can be produced by transformation with a vector which has a sequence selected from the group consisting of SEQ ID NOs: 37 – 51 and SEQ ID NO: 62.
48. (Previously presented) A foodstuff, in particular animal feedstuff, which can be produced by the method of claim 1.
49. (Previously presented) A food supplement, in particular animal feed supplement, which can be produced by the method of claim 1.
50. (Previously presented) A method for producing the foodstuff and animal feedstuff of claim 48 comprising a fermentation.
51. (Previously presented) A method for producing the food supplement and animal feed supplement of claim 49 comprising a fermentation.
52. (Previously presented) The method according to claim 35, wherein at least two products of the group consisting of foodstuff, food supplement, animal feedstuff and animal feed supplement can be obtained from a fermentation.
53. (Previously presented) The method according to claim 35, wherein the carotenoids are incorporated in the production of cosmetic, pharmaceutical, dermatological preparations, foodstuffs, food supplements, animal feedstuff or animal feed supplement.
54. (Previously presented) The method according to claim 8, wherein the hygromycin resistance gene (hph) is from *E. coli*.

55. (Previously presented) The method according to claim 33, wherein the solvent is replaced with methanol.
56. (Previously presented) The method according to claim 46, wherein the solvent is replaced with methanol.